

mechanism behind its increased amyloidogenicity: an expanded tetramer which is stabilized by a lower number of H-bonds and hydrophobic interactions. Interestingly, in the presence of T4 and lumiracoxib the structure of A25T was similar to that displayed by the wt protein. These data show that an expanded A25T tetramer with a decreased thermodynamic stability is prone to aggregate forming amyloid fibrils that trigger leptomeningeal amyloidosis. Support: CNPq and FAPERJ.

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Thermal Stability of the Extracellular Hemoglobin of *glossoscolex paulistus*: Differential Scanning Calorimetry (dsc) and Circular Dichroism (cd) Studies

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Hemoglobin of *Glossoscolex paulistus* (HbGp) in the oxy- and cyanomet-forms was studied by circular dichroism (CD) and differential scanning calorimetry (DSC). DSC experiments were performed for protein concentration 0.5 mg/ml and scan rate of 1°C/min, at pH 7.0. CD experiments were performed in the near UV to monitor the peptide region (0.2 mg/ml of protein, 195-250 nm) as well as in the Soret band spectral region (3.0 mg/ml protein, 500-250 nm) to monitor changes in the heme group environment, in the pH range 5.0-9.0. Experiments were made in the range 25-70°C. Analysis of CD data, based on a two-state thermodynamic denaturation model, allowed to obtain the fraction of denatured protein, critical temperatures as a function of pH, equilibrium constants and corresponding free energies. Cyanomet-HbGp ($T_m=65^\circ\text{C}$ at pH 7.0) is significantly more stable as compared to the oxy-form ($T_m=59^\circ\text{C}$). Our CD data suggests that the protein denatures as a whole, losing its secondary structure simultaneously for all domains of the oligomer. Critical temperatures are smaller as the pH increases in the alkaline range. On the other hand, DSC results suggest that the denaturation for oxy-HbGp is more complex, presenting low cooperativity since the endotherm could be fitted only for two components centered at 58.3 ± 0.2 and $60.6 \pm 0.1^\circ\text{C}$. For the cyanomet-form the best fit for the endotherm corresponds to three components centered at 61.6 ± 0.2 , 64.8 ± 0.2 , and $67.2 \pm 0.2^\circ\text{C}$. DSC data, in agreement with CD, also support the higher thermal stability of cyanomet-HbGp as compared to the oxy-form. Support: FAPESP, CNPq and CAPES Brazilian agencies.

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On the Thermal Stability of Extracellular Hemoglobin of *glossoscolex paulistus*: Optical Spectroscopic Studies

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Hemoglobin of *Glossoscolex paulistus* (HbGp) in the oxy- and cyanomet-forms was studied by dynamic light scattering (DLS) and optical absorption spectroscopy (OAS). At 25°C, oxy-HbGp, in the pH range 5.0-8.0, is stable presenting a mono-disperse size distribution with hydrodynamic diameter (D_h) of 27 ± 1 nm. Cyanomet-HbGp behaves in a similar way up to pH 9.0. More alkaline pH, above 9.0, induced an irreversible dissociation process, resulting in smaller D_h of 10 ± 1 nm, suggesting oligomeric dissociation. At pH 7.0, no oligomeric dissociation is observed as a function of temperature and denaturation occurs at 52°C and 57°C, respectively, for oxy- and cyanomet-HbGp. Dissociation temperatures were lower at higher pH, for both forms of HbGp. Based on the higher critical denaturation and dissociation temperatures cyanomet-HbGp is more stable than the oxy- form. Kinetic studies were performed for oxy-HbGp using UV-VIS OAS and DLS. Rate constants as a function of temperature and the activation energy (E_a) have been estimated by DLS for oxy-HbGp at pH 7.5 and 8.0, giving E_a values of 278 and 262 kJ/mol, respectively. Auto-oxidation kinetics monitored by UV-VIS at pH 8.0 in the temperature range 38-44°C is mono-exponential with an E_a value of 333 kJ/mol. Oligomeric protein dissociation promotes an increase in auto-oxidation rate and vice-versa. The present work shows that DLS is suitable to follow quantitatively the changes on the oligomerization of multisubunit proteins. Support: FAPESP, CNPq and CAPES Brazilian and FCT-MCTES Portuguese agencies.

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Geometry and Efficacy of Trp-Trp, Trp-Tyr and Tyr-Tyr Aromatic Interaction in Cross-Strand Positions of a Designed β -Hairpin

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Analysis of the impact of neighboring aromatic groups on structure can lead to improved understanding of protein folding mechanisms and stability. In this study, we examined the impact of varying aromatic interactions in cross-strand positions for Trpzip2, a β -hairpin forming peptide (Cochran et al., PNAS, 2001,

98, 5578-5583), by comparison of the interactions of Trp-Trp, Trp-Tyr and Tyr-Tyr. NMR and optical spectra (ECD, FTIR) of the original T22 peptide and its Tyr and Val-substituted mutants were analyzed to characterize their conformations and thermal stability. Cross-strand coupled Trp-Trp and Trp-Tyr pairs show unique, strong exciton bands in ECD while the Tyr-Tyr pair doesn't show any clear exciton band. The edge-to-face cross strand interaction leads to stable β -hairpin structures when Trps are at positions 2-11 or 4-9, but the alternate coupling for Trp at positions 2-9 does not lead to a stable structure. In Trpzip2 these would correspond to a more face-to-face interaction, which may contribute to the instability. When Tyr is substituted for Trp, the Trp-Trp interaction has more contribution to the peptide stabilization than does the Tyr-Tyr pair. When Tyr is substituted into position 4 and 11, the Trp-Tyr pair also has a unique geometry. These aromatic-aromatic interactions were also compared to simple hydrophobic interaction by contrasting stabilities of peptides with Val or Tyr substituted for two interacting Trp residues. Tyr is more stabilizing than Val for such substitutions which may indicate coupling of conjugated π -electron systems dominates stability. Aromatic interactions showed a stronger effect than hydrophobic interaction for stabilization. Extended kinetic studies using laser initiated T-jumps and IR detected conformational changes have helped sort out mechanistic aspects of this folding problem (Hauser et al, JACS, 2008, 130, 2984-2992).

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Effects of Mutations on Side-Specific Folding Mechanism of a Helix-Turn-Helix Protein

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Helix-turn-helix motifs are important super-secondary protein structural elements and excellent models for studying the mechanism of protein folding. We have been investigating folding of a *de novo* designed 38-residue helix-turn-helix motif α -t- α using IR spectroscopy with site-specific ¹³C isotopic editing. Our preliminary site-specific thermal unfolding data revealed that α -t- α is most stable near the centers of both α -helices, and likely unfolds from the helical termini and the loose turn region. To obtain further insights into the folding mechanism, and to investigate the roles of the individual residue-residue stabilizing interactions, we have begun mutational studies of the α -t- α protein. The mutations were designed to both destabilize and further stabilize the hydrophobic core near the helical centers. Additional mutations were designed to stabilize the helical termini and the turn/loop sequence. The overall thermodynamic stability of the α -t- α was measured using CD and IR spectroscopies. The core mutations appreciably decreased or increased the overall folding stability as intended, however, stabilizing the turn and helical termini proved to be a rather challenging task. Site-specific thermal unfolding of the mutated α -t- α were probed with IR on multiple ¹³C isotopically labeled variants of each mutant. The effects of the mutations on both the global and, in particular, local site-specific unfolding provide important clues about the stabilization of the helix-turn-helix motif by specific interactions. Although additional mutational studies are underway, thus far all the data are consistent with the proposed folding mechanism.

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Investigating Conformational Ensembles in Alanine Based Peptides Using Vibrational and Ecd Spectroscopy

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Short alanine based peptides are of interest to the protein community, due, in part to their departure from the statistical coil model. These peptides are too small to assume major secondary structures, but rather, have been found to adopt an ensemble of conformations in aqueous solution, with a predominance of PPII. However, experimental evidence suggests that the presence of charged residues might induce the sampling of multiple turn conformations, thus leading to a more compact structure of the peptide. To check this further, we measured the amide I profiles of the FTIR, Raman and VCD spectrum of H-(AKAAW)-OH, and subsequently simulated the vibrational spectra using an excitonic coupling model, with NMR coupling constant and end-to-end distance constraints. We included multiple conformations: PPII, β -strand, $_R$ helix, $_L$ helix, and turns. The alanine residues experienced a high propensity for PPII structure, ~70%, while ~20% for β -strand conformations and smaller percentages for other coil structures. Lysine, however showed a larger propensity for β -strand ~30% than the alanine residues, but the PPII content for lysine is still high (~42%). We obtained an end-to-end distance of 10Å, which is in accordance with FRET measurements of the end to end distance of